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EFFECT OF BENTONITE ON THE ENZYMATIC ACTIVITY OF SOIL MICROFLORA

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16. Abstract

The presence of bentonite (0.1-2%) in a sandy and liquid medium with proteins stimulates the forming of neutral and alkaline proteases in mixed bacterial cultures and catalase activity. In the case of a concentration above 5% in the nutrient medium, a depressive influence predominates. In the presence of bentonite, the accumulation curves of enzymes reach their maxima earlier than they do in the control medium. Proteases absorbed by 15% bentonite are inactivated by 75%.

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EFFECT OF BENTONITE ON THE ENZYMATIC ACTIVITY OF SOIL MICROFLORA

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The clay fraction of the soil is considered to be exceptionally important in the determination of soil characteristics. It may have an indirect effect on microbial activity by way of the adsorption of nutrients, microbial metabolites, autolysis products, and a direct effect by way of the action of colloidal particles with microbe cells. /209*

An extensive literature on the ability of clay minerals to fix various organic compounds and thus prevent their decomposition to a certain extent, which was recently summarized in Calvet's monograph (1963), is available. From the experimental tests, the results obtained by Talibudeen (1965), Pinck, Dyal, and Allison (1954), and also McLaren et al (1958) are interesting. It follows from these tests that organic compounds, primarily proteins, can be bonded by clay minerals in different ways which depend on the mineral-to-protein ratio. The possibility of the proteins concentrating on the surface of the clay mineral simultaneously with proteolytic enzymes is also considered, which, under certain conditions, as shown by Esterman et al. (1959), makes more vigorous decomposition possible.

Durand (1966) studied the fixation of purine and pyrimidine bases with bentonite. He found out that in an acidic medium the cations formed by the bases are exchanged by the bentonite ions H^+ and Al^{3+}. Urikase may also be bonded simultaneously with uric acid, which reduces its activity. The enzyme also

*Numbers in the margin indicate pagination in the foreign text.

acts in the absorbed state, and it need not first be squeezed out into the solution.

The tests that were described, which studied the adsorption of organic compounds on clay minerals, were predominantly carried out under abiotic conditions in which the decomposition was obtained by adding appropriate enzymatic preparations. The situation is more complicated in the presence of microbes. In addition to the possible adsorption and desorption of the organic compounds, among these also enzymes, their accessibility to microbes also plays a role. The microbes react to this state in a manner which is different from the decomposition of the organic compound itself. In addition, the microbes may also be adsorbed; hence, their vital activity is directly affected. Studies on the participation of microbes in the processes that were described are comparatively scanty. Therefore, we present some of our own results as a contribution to the solution of this problem.

Material and Methods /210

The effect of bentonite on proteolytic microflora was studied in Petri dishes filled with sand to which basic mineral compounds were added (mineral nutrients from Thornton soil) and proteins, most often in a 1% concentration. Gelatine, casein, ovalbumin, or dried egg albumin was used as the protein. In later experiments we worked with a well-shaken liquid medium composed of a solution of protein, mineral salts and bentonite in different concentrations. A culture suspension was used for the inoculation and incubation took place at 28°C or at laboratory temperature. The enzymatic activity of the microflora was studied in the tests every day for a period of 1 week.

The activity of the proteases was measured using the colorimetric method described earlier (Ambroz, 1966) and the catalase activity was determined manganometrically in the usual way.

Branan bentonite, whose main component is montmorillonite with kaolinite inclusions was used.

Results

In the bentonite tests two groups of bacterial proteases were selected, of which one has optimum pH value of 6.5 and is referred to as the neutral protease (NP), and the other, due to the higher optimal pH value 8.5, is referred to as the alkaline protease (AP). After several tests were made, the effect of the bentonite was as follows: At low concentrations between 0.1-2%, it stimulated the microbial protease production considerably (Figs. 1 and 2).

When the bentonite content was increased in the medium /211
to 2-5%, its effect was not always the same; in high concentrations, above 10%, the protease and catalase activity was often depressed. In the medium with bentonite, the maximum enzymatic activity occurred earlier, even in many cases when the bentonite was already acting as a depressant. The results of analyses of the enzymatic activity are summarized in Table 1.

The stimulating or possibly depressing effect manifested /212
itself both in the liquid and sandy medium. I did not assume that it was only due to the mechanical contact of the bentonite particles with the microbes. I focused my attention primarily on the adsorbing properties of the bentonite.

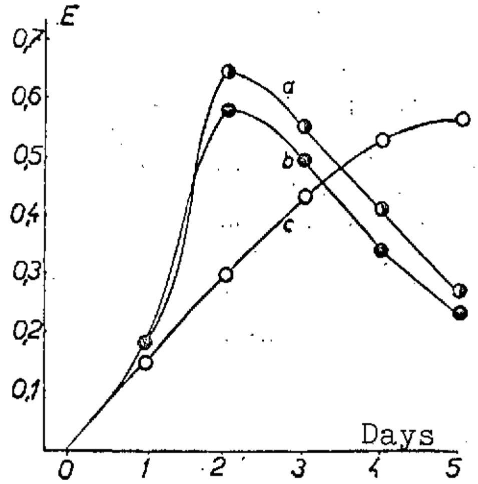


Fig. 1. Activity of neutral proteases during the incubation of a liquid medium and 1% of gelatine with the addition of bentonite (a = 0.1%; b = 1%; c = control sample without bentonite).

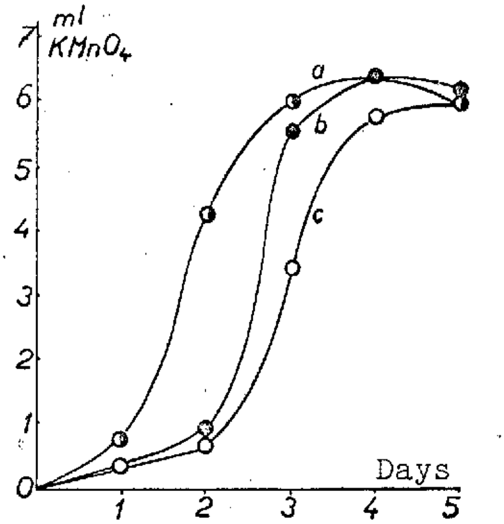


Fig. 2. Activity of the catalase in a liquid medium with 1% of gelatine and the addition of bentonite. a = 0.1%; b = 1%; c = control sample without bentonite.

TABLE 1. EFFECT OF BENTONITE ON ENZYMATIC ACTIVITY OF MICROBES (AS A PERCENTAGE OF POSITIVE RESULTS).

	Enzyme Stimulation		
	0.1-2% Bentonite	2-4% Bentonite	5-20% Bentonite
Neutral proteases	93% (15 tests)	66% (6 tests)	28% (7 tests)
Alkaline proteases	87% (15 tests)	50% (6 tests)	14% (7 tests)
Catalase	93% (15 tests)	83% (6 tests)	28% (7 tests)
	Enzyme Maximum Lead		
Neutral proteases	100% (14 tests)	86% (7 tests)	43% (7 tests)
Alkaline proteases	85% (14 tests)	71% (7 tests)	30% (7 tests)
Catalase	93% (14 tests)	100% (7 tests)	71% (7 tests)

In the next series an attempt was made to study the dynamics of the proteases and their fractionation into free proteases and proteases bonded with bentonite. The adsorbed proteases were determined indirectly from the difference in the total activity in the samples with bentonite and the activity after it was removed (Figs. 3 and 4).

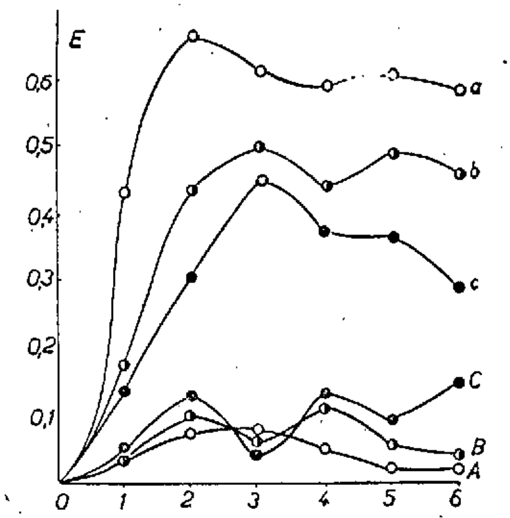


Fig. 3. Dynamics of free (a,b,c) and absorbed (A,B,C) neutral proteases in a liquid medium with 0.8% egg albumin. Bentonite added: a,A - 1%; b,B - 10%; c,C - 20%.

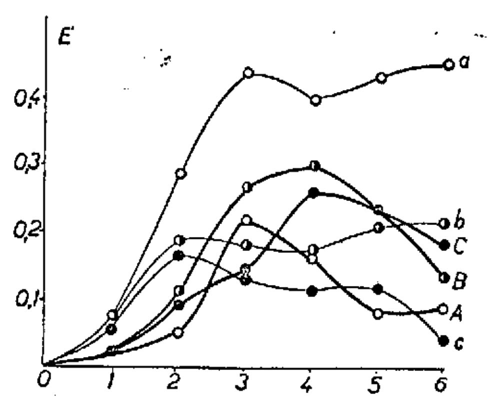


Fig. 4. Dynamics of free (a,b,c) and absorbed (A,B,C,) alkaline proteases in a liquid medium with 0.8% egg albumin. Bentonite added: a,A - 1%; b,B - 10%; c,C - 20%.

It follows from the results that the activity of the free proteases drops with the increase in the bentonite concentration, whereas the activity of the adsorbed proteases increases. The alkaline proteases are bonded more strongly by bentonite than the neutral proteases.

The calculations given in Table 2 show how strongly bentonite inactivates the proteases.

TABLE 2. CALCULATION OF THE ADSORPTION AND INACTIVATION OF PROTEASES BONDED BY 15% BENTONITE.

	Neutral Proteases %	Alkaline Proteases %
A. Original enzyme activity	100	100
B. Activity after 15% bentonite was added	73	59
C. Activity after bentonite was extracted	63	45
D. Adsorbed by bentonite (A - C)	37	55
E. Adsorbed enzyme activity ((B - C)/D)100	27	25
F. Inactivation of adsorbed enzymes (A - E)	73	75

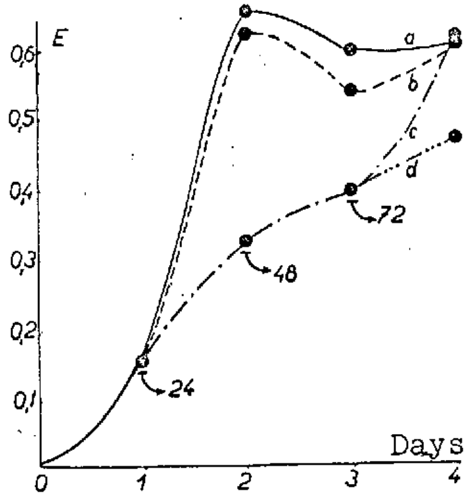
In the next test, using a liquid medium with a 5% bentonite content, the bentonite was gradually removed from a considerable part by moderate centrifugation after 24, 48 and 72 hours, and the medium was incubated further. Although this measure removed a part of the microflora, nevertheless the enzymatic activity increased after centrifugation, and it attained the values of the control sample without bentonite. Evidently the depressing effect was not due to the release of certain substances from the bentonite during the exchange, but rather to its absorption capacity.

Discussion

The data about the effect of clay materials on the biochemical activity of microbes are highly contradictory. Data on the depressing effect of adsorbents on microbes are available (Hattori and Furusaka, 1961; Durand, 1966; Lahav and Keynan, 1962, Zvyagintsev,1959). On the other hand, many authors encountered the stimulating effect. The favorable effect of low bentonite concentrations on the fixation capacity of azotobacteria is mentioned, for example, by Macura and Pavel (1959), and on the development of various groups of microbes, by Filip (1967).

For the time being, it is difficult to say what the mechanism for the stimulating effect of the clay minerals is, since it need not necessarily occur only with the adsorption phenomena, but also occurs in the presence of substances that are only slightly adsorbed.

Under the conditions described in this study, another possibility should be mentioned in addition to the concentration of the substrate and enzymes on the surface of the clay materials. In the presence of bentonite, a medium with a relatively lower content of accessible nutrients is formed /213



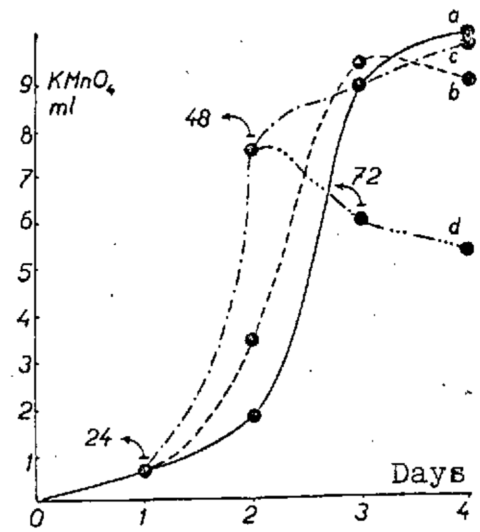


Fig. 5. Effect of bentonite separation (5%) on the dynamics of neutral proteases. a - Control medium without bentonite; bentonite separated ; b - after 24 hours of incubation; c - after 48 hours of incubation; d - after 72 hours of incubation.

Fig. 6. Effect of bentonite separation (5%) on the dynamics of the catalase. a - Control medium without bentonite; bentonite separated ; b - after 24 hours of incubation; c - after 48 hours of incubation; d - after 72 hours of incubation.

as the result of the partial adsorption, and the reaction of the microbes to this state may be higher enzymatic production. For higher bentonite concentrations, the more intensive enzyme production would be masked by their strong adsorption and inactivation. Some of the results point to this possibility, but in several cases, even comparatively high bentonite concentrations stimulated the microbes to such an extent that the depressing effect did not occur.

The lead in the enzyme maximum in a medium with bentonite is probably related to the phenomenon described by Novakova (1968), who determined a shorter lag phase in her study on the effect of bentonite on the E. coli growth curve. Hattori and Furusaka (1961) describe a similar phenomenon in Azotobacter agile, and they think that it is caused by the bonding of a certain cellular nucleotide fraction by the absorbent causing the lat ent phase.

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